The C, V and W proteins of Nipah virus inhibit minigenome replication

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Nipah virus (NiV) is a recently emergent, highly pathogenic, zoonotic paramyxovirus. NiV was first identified in Malaysia in 1998 during an outbreak of neurological and respiratory disease in swine, which resulted in severe febrile encephalitis in humans who had contact with infected pigs (Chua et al., 2000). NiV has been associated with subsequent outbreaks of fatal, febrile encephalitis in Bangladesh and India between 2001 and 2005 (Hsu et al., 2004; Luby et al., 2006). Molecular characterization of NiV revealed that it was related closely to Hendra virus, another zoonotic paramyxovirus, which had emerged in Australia in 1994 (Chua et al., 2000; Harcourt et al., 2000, 2001). These two viruses constitute the recently recognized genus Henipavirus (Mayo, 2002).

The genome of NiV is 18 246 nt in length and contains six transcription units encoding six viral structural proteins (3’-N-P-M-F-G-L-5’) and three predicted non-structural proteins, C, V and W (Harcourt et al., 2000). As in other paramyxoviruses, the C protein of NiV is expressed from an alternative open reading frame (ORF) within the phosphoprotein (P) gene, whereas the V and W proteins are expressed by RNA editing (Lamb & Kolakofsky, 2001). At a unique, highly conserved RNA-editing site in the P gene, the polymerase protein (L) inserts a single, non-templated G residue that results in a frame shift and the expression of the V protein. Insertion of two non-templated G residues results in expression of the W protein (Harcourt et al., 2000). The sequences of the P proteins of paramyxoviruses are not well conserved (Baron et al., 1993) and, whilst a V protein is not expressed by all paramyxoviruses, the unique carboxyl-terminal domain of V is more conserved than the amino-terminal domain shared with P (Galinski et al., 1992; Matsuoka et al., 1991; Witko et al., 2006). The C, V and W proteins of paramyxoviruses are known to play multiple roles in the viral life cycle, functioning as interferon antagonists (Didcock et al., 1999; Goodbourn et al., 2000; He et al., 2002; Komatsu et al., 2004; Lin et al., 2005; Nanda & Baron, 2006; Ohno et al., 2004; Park et al., 2003; Poole et...
As very little is known about the replication of the henipaviruses, the goal of this research was to investigate the roles that the C, V and W proteins of NiV play in viral transcription and replication. Here, we demonstrate that the C, V and W proteins of NiV inhibit NiV minigenome replication. The NiV C, V and W proteins also inhibited replication of a measles virus (MV) minigenome, and the C and V proteins of MV inhibited NiV minigenome replication. However, the accessory proteins of NiV and MV were not able to inhibit human parainfluenzavirus 3 (hPIV3) minigenome replication. Conversely, the C protein of hPIV3 inhibited minigenome replication of both NiV and MV. These findings suggest that these divergent viral proteins share common functional properties, perhaps through interaction with a common host-cell protein(s).

**METHODS**

**Molecular techniques.** Standard DNA manipulations were performed according to methods described by Sambrook & Russell (2001). All plasmids were cloned and prepared by using Escherichia coli strain DH5α purified and confirmed by sequencing. The coding sequences of all inserts were confirmed by sequencing. Sequence reactions were performed by using a cycle-sequencing reaction with fluorescent dye terminators (Perkin-Elmer Applied Biosystems) and the reaction products were analysed by an ABI 3100 (Perkin-Elmer Applied Biosystems) automatic sequencer. Sequence data were analysed with Sequencher software (Gene Codes Corporation).

**Cells.** Baby hamster kidney cells constitutively expressing T7 polymerase (BHK/ark/T7 cells; Buchholz et al., 1999) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 10% tryptase phosphate broth (BD Biosciences), 400 μg G418 ml⁻¹, 4 mM L-glutamine, 50 U penicillin ml⁻¹ and 50 μg streptomycin ml⁻¹ (Mediatech).

**Derivation of plasmids.** Plasmids encoding the N, P and L proteins of NiV and the NiV minigenome have been described previously (Halpin et al., 2004). hPIV3 plasmids used were a kind gift from Dr Sue Moyer (University of Florida, Gainesville, FL, USA) and have been described elsewhere (Smallwood & Moyer, 2004); plasmids expressing the N, P and L proteins of MV, the MV CAT minigenome and the C proteins of the Zagreb vaccine strain and CAM-70 strain have been described previously (Bankamp et al., 2005).

Plasmids expressing the C, V and W proteins of NiV were generated by amplifying the relevant ORFs from existing plasmids obtained from Dr Brian Harcourt (Centers for Disease Control and Prevention, Atlanta, GA, USA) and each protein was tagged at the amino-terminal domain of the protein with an HA (hemagglutinin) epitope tag to enable confirmation of protein expression. The plasmid NiV/HA/C was produced by amplifying the entire C protein ORF with an attached amino-terminal HA tag by using the primers HA/C/F (5'-GATCGAGGCTCACCATGTACCTACAGGATTTAGCGTATGATGG CCTCAAAATTATTGAC-3') and HA/C/R (5'-GATCCGCTGATGTTCCGATGCATCC-3'). NiV/H/V and NiV/H/W were constructed by using the primers HA/V/F (5'-GATCGAGGCTCACCATGTACCTACAGGATTTAGCGTATGATGGCCTCAAAATTATTGAC-3') and HA/V/R (5'-GATCCGCTGATGTTCCGATGCATCC-3'), whilst NiV/H/WUtilized primers HA/C/F and HA/C/R (5'-GATCCGAGCTCACCATGTACCTACAGGATTTAGCGTATGATGGCCTCAAAATTATTGAC-3') and NiV/H/V/C was produced by amplifying the entire C protein ORF with an HA (haemagglutinin) epitope tag to nickel (Ni) affinity purification.

**Protein expression detected by radioimmunoprecipitation**. BHK/ght/17 cells in 35 mm dishes were transfected with the desired plasmid constructs by using LT-1 transfection reagent (Mirus) according to the manufacturer’s instructions. Cells were labelled approximately 36 h post-transfection with [35S]methionine in methionine-free medium (ICN) for a period of 2 h. Cells were lysed in RIP buffer [150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-Cl (pH 8.0)] and each lysate supernatant was immunoprecipitated with 2.5 μl mouse antiserum specifically recognizing the P, C, V or W proteins of NiV or rabbit antiserum to the HA tag overnight at 4 °C, with rotation. Precipitation using GammaBind G–Sepharose (Amersham Pharmacia Biotech) was then performed; protein complexes were separated by SDS-PAGE and bands were visualized by autoradiography.

**Replication assay and CAT ELISA.** BHK/ght/17 cells were transfected with the desired plasmid constructs in Opti-MEM medium (Invitrogen) by using LT-1 transfection reagent (Mirus) according to the manufacturer’s instructions. Total amounts of transfected DNA were kept constant by including additional empty-vector plasmid DNA where applicable. Details of the NiV minigenome replication assay have been described elsewhere (Halpin et al., 2004), as have details of the hPIV3 (Smallwood & Moyer, 2004) and MV (Bankamp et al., 2005) minigenome replication assays. Cytoplasmic extracts were prepared 36–42 h post-transfection in 1 ml lysis buffer (part of the CAT ELISA kit; Roche). To ensure comparable results, the amount of harvested cytoplasmic extracts used per sample was adjusted for protein concentration. The total protein concentration of the lysate supernatants and the concentration of CAT reporter protein in the samples were determined according to the manufacturer’s instructions. Details of the NiV minigenome replication assay have been described elsewhere (Halpin et al., 2004), as have details of the hPIV3 (Smallwood & Moyer, 2004) and MV (Bankamp et al., 2005) minigenome replication assays. Cytoplasmic extracts were prepared 36–42 h post-transfection in 1 ml lysis buffer (part of the CAT ELISA kit; Roche). To ensure comparable results, the amount of harvested cytoplasmic extracts used per sample was adjusted for protein concentration. The total protein concentration of the lysate supernatants and the concentration of CAT reporter protein in the samples were determined according to the manufacturer’s instructions. Details of the NiV minigenome replication assay have been described elsewhere (Halpin et al., 2004), as have details of the hPIV3 (Smallwood & Moyer, 2004) and MV (Bankamp et al., 2005) minigenome replication assays. Cytoplasmic extracts were prepared 36–42 h post-transfection in 1 ml lysis buffer (part of the CAT ELISA kit; Roche). To ensure comparable results, the amount of harvested cytoplasmic extracts used per sample was adjusted for protein concentration. The total protein concentration of the lysate supernatants and the concentration of CAT reporter protein in the samples were determined.
Micrococcal nuclease-treated RNA that was additionally treated with DNase I (TURBO; Ambion), or poly(A)^+ selected mRNA, was used in each 25 μl reaction volume. One-step RT-PCR was performed in duplicate reactions using reagents supplied in a TaqMan Gold RT-PCR kit (Applied Biosystems) as described previously (Hummel et al., 2006). Briefly, a reverse-transcription step at 48 °C for 30 min was followed by DNA-polymerase activation at 95 °C for 10 min and 40 PCR cycles of 95 °C for 15 s and 60 °C for 1 min. Negative-control reactions included samples run without the addition of template (NTC) or reverse transcriptase.

RNA samples were considered positive only if threshold cycle (Ct) values were <40. CAT RNA was not detected in the cell control, which contained transfection reagent only, or the negative-control reactions (Ct=40). The comparative Ct method (ΔΔCt) was used for the relative quantification of gene expression, using CAT as the target and normalized to the endogenous reference, RNase P (ΔCt=CtCAT – CtRNaseP). Fold change (2^−ΔΔCt) in CAT RNA was determined by subtracting the ΔCt value of the baseline condition, in which the NiV L plasmid was omitted from the replication assay, from each sample (ΔΔCt=ΔCt−ΔCtNTC).

RESULTS

The C, V and W proteins of NiV inhibit NiV minigenome replication

To study the abilities of the C, V and W proteins of NiV to regulate viral transcription and/or replication, we used an existing NiV minigenome replication assay (Halpin et al., 2004). Plasmid DNA expressing NiV C, V or W was co-transfected with plasmid DNAs expressing the N, P and L proteins of NiV, in addition to the plasmid containing the NiV CAT minigenome. In the standard replication assay, expression of C from the plasmid DNA expressing P was silenced and the lack of expression of C from plasmids encoding NiV P, V and W was verified by immunoprecipitation (data not shown). In these experiments, the amount of CAT produced from the positive control, consisting of plasmid DNAs expressing the NiV minigenome and the N, P and L proteins, was set at 100 % and the total amount of plasmid DNA in each transfection was kept constant by the addition of empty-vector plasmid DNA. Inclusion of 2 μg plasmid DNA encoding the C, V or W proteins of NiV caused a reduction of approximately 70, 80 and 90 %, respectively, in the amount of CAT reporter protein expressed compared with that of the positive control (Fig. 1a); the inhibitory effect was dose-dependent (range, 0.5–2 μg) for each of the proteins (Fig. 1b, c, d).

Inhibition of NiV minigenome replication by V and W deletion mutants

To identify which regions of the V and W proteins of NiV were required for the inhibitory effect on NiV minigenome replication, plasmids expressing amino- and carboxyl-terminal deletion mutants of each of the proteins were generated as described in Methods. Each of the deletion mutants was HA-tagged at the amino terminus to enable detection of protein expression. Full-length constructs expressing HA-tagged C, V

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Triton X-100, 10 mM Tris (pH 7.5), 10 mM NaCl, 1.5 mM MgCl₂, 10 mM CaCl₂, 4.5 kIU aprotinin ml⁻¹ (Roche), 100 μg micrococcal nuclease S7 ml⁻¹ (Roche). Cell lysates were transferred to a 1.5 ml tube and incubated for 30 min at 30 °C. RNA was then extracted by using Trizol LS (Invitrogen). Trizol LS (750 μl) was added to each sample and incubated for 5 min at room temperature; 200 μl chloroform was then added and the contents of the tube were mixed briefly, then incubated for 3 min at room temperature. Samples were then centrifuged at 12 000 r.p.m. for 15 min at 4 °C. The supernatant was transferred to a fresh tube and 1 μl glycol blue, 1 μl yeast RNA, 8 μl RNase-free water and 500 μl 2-propanol were added. Samples were incubated for 10 min at room temperature, after which they were centrifuged at 15 000 r.p.m. at 4 °C for 20 min. The supernatant was discarded and the resulting RNA pellet was washed with 1 ml cold 75 % ethanol. The sample was then centrifuged at 12 000 r.p.m. for 1 min. Following this incubation, 30 μl formaldehyde loading buffer (Ambion) was added to each sample and the sample was incubated at 68 °C for 10 min.

Poly(A)^+ selected mRNA purification. An Oligotex Direct mRNA mini kit (Qiagen) was used according to the manufacturer’s recommendations. Final RNA pellets were resuspended in 10 μl RNase-free water and incubated at 55 °C for 10 min. A 30 μl volume of formaldehyde loading buffer (Ambion) was added to each sample and samples were incubated at 68 °C for 10 min.

RNA probe design and synthesis. Digoxigenin (DIG)-labelled CAT RNA probes were synthesized by PCR using primers for negative-sense probes as described previously (Bankamp et al., 2002). An SP6/7 Transcription kit and a DIG Northern starter kit (both from Roche) were used according to the manufacturer’s specifications and the marker probe was generated with the Millennium marker probe template (Ambion).

Northern blot analysis. RNA samples prepared as described above were analysed with RNA Millennium size markers (Ambion) on a 1.5 % agarose formaldehyde gel. After electrophoresis at 100 V for 4 h in MOPS–EDTA acetic buffer [0.1 M (pH 7.5), 0.15 M NaCl, 0.1 M Tris/HCl (pH 9.5), 0.1 M NaCl, 10 mM Tris/HCl (pH 9.5), 0.1 M maleic acid, 0.15 M NaCl] for 90 min in standard saline citrate (SSC) for 90 min and then washings performed as described previously (Bankamp et al., 2005). The blots were then crosslinked using the AUTO setting on a UV Stratalinker 1800 (Stratagene). Prehybridization and hybridization to negative-sense probe template (Ambion). From Roche) were used according to the manufacturer’s specifications, which contained transfection reagent only, or the negative-control reactions (Ct=40). The comparative Ct method (ΔΔCt) was used for the relative quantification of gene expression, using CAT as the target and normalized to the endogenous reference, RNase P (ΔCt=CtCAT – CtRNaseP). Fold change (2^−ΔΔCt) in CAT RNA was determined by subtracting the ΔCt value of the baseline condition, in which the NiV L plasmid was omitted from the replication assay, from each sample (ΔΔCt=ΔCt−ΔCtNTC).

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and W proteins were also generated to test whether the inclusion of the HA tag at the amino terminus of each of the proteins affected their function within the minigenome system. Expression of the NiV C, V and W full-length and truncated proteins was confirmed by immunofluorescence assay and immunoprecipitation (data not shown).

Each of the full-length HA-tagged C, V and W proteins inhibited CAT expression to approximately the same extent as the untagged versions of the full-length C, V and W proteins (Fig. 2). Plasmids expressing aa 1–220 of the 407 aa shared amino-terminal domain of V and W, or a protein containing the 52 aa unique carboxyl-terminal domain of V (aa 221–457), inhibited CAT expression from the NiV minigenome to approximately the same extent as the intact V and W proteins (Fig. 2). A plasmid expressing a protein containing the 47 aa unique carboxyl-terminal domain of W (aa 221–451) inhibited CAT expression by approximately 30%, whilst the full-length W inhibited reporter gene expression by 80–90% (Fig. 2). Deletion mutants that encoded the first 83 aa or aa 84–166 of the C protein were difficult to detect by immunoprecipitation. The minimal inhibitory effects that these deletion mutants had on CAT expression could have been a result of instability (data not shown).

**Heterologous inhibition of paramyxovirus minigenome replication**

Due to the lack of sequence similarity and conservation among the non-structural proteins from different genera of the family *Paramyxoviridae*, it was expected that the NiV C, V and W proteins would not inhibit the replication of other paramyxoviruses. To determine the specificity of inhibition of CAT expression from the NiV minigenome by NiV C, V and W proteins, the C and V proteins of MV were tested for their effects on NiV minigenome expression. Surprisingly, the C proteins from two strains of MV, CAM-70 (CAM) and Edmonston-Zagreb (ZAG), inhibited CAT expression from the NiV minigenome by 50 and 80%, respectively, compared with 68% inhibition by the NiV C protein, 78% by the NiV V protein and 85% inhibition by the NiV W protein (Fig. 3a). Furthermore, the V protein of CAM-70 also inhibited CAT expression by approximately 70%. The F protein of the Edmonston strain of MV (MV F) had no inhibitory effect (Fig. 3a) and was included to demonstrate that inhibition was limited to viral accessory proteins. These data were unexpected, due to the high level of sequence divergence between the genera *Henipavirus* and *Morbillivirus*.
To investigate the heterologous inhibition of paramyxovirus minigenome replication further, minigenome replication assays for MV (Edmonston strain) and hPIV3 were used. The C, V and W proteins of NiV inhibited CAT expression from the MV minigenome by 30–50%, whilst MV C inhibited reporter-gene expression by 90% (Fig. 3b). It has already been shown that the C protein of hPIV3 inhibits hPIV3 minigenome replication (Malur et al., 2004; Smallwood & Moyer, 2004). The C, V and W proteins of NiV and the C protein of MV had no inhibitory effect on CAT expression from the hPIV3 minigenome (Fig. 3c). However, the C protein of hPIV3 inhibited expression from both the NiV and MV minigenomes by 90 and 70%, respectively, levels comparable to those of the homologous accessory proteins, MV C and NiV W (Fig. 4a, b). This suggests that there may be more than one mechanism by which the C, V and W proteins of paramyxoviruses regulate expression from the minigenome, as hPIV3 C was able to inhibit CAT expression from minigenomes representing all three genera tested with equal effect. In contrast, NiV C, V and W and the MV C and V proteins were more restricted in their inhibitory activities.

**NiV C, V and W inhibit both transcription and replication**

The inhibition observed in the NiV replication assay with the NiV accessory proteins was evaluated further to determine whether it correlated with a reduction in both CAT mRNA and encapsidated minigenome RNA populations. Northern blots showed that the amount of CAT mRNA produced by the NiV minigenome was consistently lower than the amount of RNA produced by the...
minigenome of MV (Fig. 5a) and, in the presence of NiV C, V or W proteins, CAT mRNA was undetectable (Fig. 5b). Real-time RT-PCR confirmed that comparable amounts of cellular RNA were loaded in each lane in Fig. 5(b) (data not shown).

Real-time RT-PCR analysis was performed on the mRNA samples, as it is a more sensitive method than Northern blotting for detection of low levels of CAT RNA. The amount of transfected plasmid DNA was kept constant by the addition of pTM1 vector. CAT protein concentration in cytoplasmic extracts was measured by ELISA and CAT expression in the absence of inhibiting proteins was set to 100 %. Experiments were performed at least three times and results of a single representative experiment are shown. Error bars represent SD between duplicate samples.

DISCUSSION

It is widely acknowledged that the non-structural proteins encoded by many members of the family Paramyxoviridae play multiple roles in the viral life cycle, from regulating viral transcription and replication to modulating the host immune response. To determine whether the C, V and W proteins of the henipavirus NiV also possess the ability to regulate replication of the viral genome, a plasmid-based minigenome assay was used. A similar approach has been used to study the regulation of viral genome replication for multiple paramyxoviruses, including MV (Bankamp et al., 2002, 2005; Reutter et al., 2001; Sidhu et al., 1995), hPIV3 (Durbin et al., 1997; Malur et al., 2004; Smallwood & Moyer, 2004), Sendai virus (SeV) (Tapparel et al., 1997), simian virus 5 (SV5) (Lin et al., 2005), respiratory syncytial virus (Atreya et al., 1998), rinderpest virus (Brown et al., 2005), peste-des-petits-ruminants virus (Bailey et al., 2007), J-virus and Beilong virus (Magoffin et al., 2007). We demonstrated that NiV C, V and W proteins inhibit CAT expression from the NiV minigenome in a dose-dependent manner. The synthesis of both mRNA and genomic RNA in the replication assays.

Inhibition of NiV minigenome replication in the absence of inhibiting proteins was set to 100 %. Experiments were performed at least three times and results of a single representative experiment are shown. Error bars represent SD between duplicate samples.

Fig. 4. The C protein of hPIV3 inhibits CAT expression from the minigenomes of both NiV and MV. BHK/sr/T7 cells were transfected with plasmid DNAs expressing minigenome and N, P and L proteins of NiV (a) or MV (b). In addition, MV C, NiV C, V or W, or hPIV3 C were added to the transfections as indicated. The ‘cells only’ control contained transfection reagent only, but no plasmid DNA. The amount of transfected plasmid DNA was kept constant by the addition of pTM1 vector. CAT protein concentration in cytoplasmic extracts was measured by ELISA and CAT expression in the absence of inhibiting proteins was set to 100 %. Experiments were performed at least three times and results of a single representative experiment are shown. Error bars represent SD between duplicate samples.

Fig. 5. Northern blot detection of CAT mRNA produced in the minigenome replication assay for NiV. BHK/sr/T7 cells were either transfected with plasmid DNAs expressing minigenomes of NiV or MV together with the homologous N, P and L proteins (a) or plasmid DNAs expressing NiV minigenome and N, P and L proteins in addition to the C, V or W proteins of NiV (b). In the negative control, the plasmid DNA expressing the homologous L protein was omitted. For (b), the amount of transfected plasmid DNA was kept constant by the addition of pTM1 vector. Forty-two hours after transfection, poly(A)+-selected RNA was prepared and separated on a formaldehyde–agarose gel, transferred to a nylon membrane and hybridized with a DIG-labelled, negative-sense, CAT-specific RNA probe. Chemiluminescence was detected by autoradiography.
be a result of inhibition of replication or the result of another, independent mechanism.

The observed inhibitory effect of NiV C on NiV minigenome replication is consistent with previously published observations for other paramyxoviruses. MV C has previously been shown to downregulate reporter-gene expression from minigenome constructs (Bankamp et al., 2002; Reutter et al., 2001). Like the C proteins of the respiroviruses hPIV3 (Smallwood & Moyer, 2004; Malur et al., 2004) and SeV (Curran et al., 1992; Grogan & Moyer, 2001; Kato et al., 2002, 2004), MV C inhibits viral RNA transcription and replication specifically (Bankamp et al., 2005). In the case of MV, the C protein binds the large (L) protein, the major subunit of the RNA-dependent RNA polymerase complex (Smallwood & Moyer, 2004), and for SeV, the C–L interaction is required for the inhibitory effect on viral RNA synthesis (Grogan & Moyer, 2001). It is possible that NiV C is acting in a similar manner by interacting with NiV L. Such a potential interaction warrants further investigation.

The V proteins of several paramyxoviruses play a role in regulating viral replication. Recombinant paramyxoviruses lacking V proteins, including rinderpest virus (Baron & Barrett, 2000), SeV (Kato et al., 1997) and MV (Tober et al., 1998), produced increased levels of genomic RNA, mRNA and viral proteins. In addition, the V protein of SeV inhibited the replication of defective interfering particles (Curran et al., 1991; Horikami et al., 1996), and the V protein of SV5 inhibited viral transcription and replication (Lin et al., 2005). For a number of paramyxoviruses, it has been postulated that the mechanism by which V controls viral replication may be through a direct protein–protein interaction with the nucleocapsid (N) protein (Curran et al., 1991; Horikami et al., 1996; Lin et al., 2005; Svenda et al., 2005; Sweetman et al., 2001). However, recent studies with the V protein of MV discounted the possibility that a V–N interaction is responsible for the inhibitory effect (Witko et al., 2006). The ability of the V protein of MV to bind RNA correlated directly with the ability of MV V to inhibit minigenome replication (Parks et al., 2006), although the mechanism remains to be determined.

A recent publication (Witko et al., 2006) reported results similar to those from our study, as it identified two regions of the MV V protein, one at the amino terminus and one at the carboxyl terminus, that were independently capable of inhibiting CAT expression from the minigenome of MV. Furthermore, we observed that the first 220 aa acids of the shared amino-terminal domain of V/W and a protein containing the unique region of V produced levels of inhibition equivalent to those measured for full-length V and W, whilst a protein containing the unique carboxyl-terminal domain of W was only able to inhibit replication by 30%.

This is the first report of heterotypic inhibition of paramyxovirus minigenome replication by the accessory proteins C, V and W. Despite the lack of amino acid conservation between the C, V and W proteins of NiV, MV and hPIV3, the hPIV3 C protein was able to inhibit NiV and MV minigenome replication, although not to the same extent as the homologous proteins. The C, V and W proteins of NiV and MV inhibited both the homologous and heterologous replication assays, although the levels of heterologous inhibition were not as great as the levels of inhibition achieved with homologous proteins. Taken together, these findings suggest that these divergent viral proteins may share common functional properties. The mechanism(s) of the cross-genus inhibition is currently under investigation and it is likely that conserved host-cell proteins may be involved in a common inhibitory pathway.

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REFERENCES


Table 1. Relative amount of CAT gene expression in the NiV minigenome replication assay

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<th>Poly(A)†</th>
<th>Micrococcal nuclease-resistant RNA†</th>
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<tr>
<td>NiV +ve</td>
<td>4.43</td>
<td>2.56 ± 0.03</td>
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<tr>
<td>NiV C</td>
<td>0.08</td>
<td>0.07 ± 0.02</td>
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<tr>
<td>NiV V</td>
<td>0.20</td>
<td>0.22 ± 0.12</td>
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<tr>
<td>NiV W</td>
<td>0.05</td>
<td>0.12 ± 0.01</td>
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*2−ΔAC, values calculated from replicate transfection wells run in duplicate in real-time RT-PCR.

†2−ΔAC, values calculated from replicate transfection wells run in duplicate in real-time RT-PCR; mean ± SD from two separate experiments.
Inhibition of NiV minigenome replication


